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RESEARCH NOTE

Comparison of strand displacement and ligase chain amplification for detection of *Chlamydia trachomatis* infection in urogenital specimens

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ABSTRACT

Two amplification tests for the diagnosis of *Chlamydia trachomatis* infection, namely the ligase chain reaction (LCx) and the strand displacement assay (ProbeTec), were compared using samples from 1183 patients at sexually transmitted disease

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clinics. The overall prevalence of positive results was 8.0%, with agreement between the two assays of 98.8%. For endocervical, urethral and male urine samples, agreement was 99.3%, 99.4% and 97.7%, respectively. For ten discrepant samples, alternative amplification assays suggested that the LCx and ProbeTec assays gave erroneous results in six and four cases, respectively. Inhibition of amplification was observed with three (0.25%) urine specimens.

Keywords *Chlamydia trachomatis*, DNA amplification, ligase chain reaction, sexually transmitted disease, strand displacement assay

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Chlamydia trachomatis is the most common bacterial cause of sexually transmitted disease in the Western world [1]. Untreated, acute chlamydial infections, such as cervicitis and urethritis, may be complicated by pelvic inflammatory disease and infertility, chronic abdominal pain, and reactive arthritis [2]. Since both symptomatic and asymptomatic cases may be at equal risk of suffering these complications, the important role of early laboratory diagnosis, screening programmes and contact tracing is well-recognised [3].

In Sweden, following a decline during the previous decade, the incidence of *C. trachomatis* infection increased from 13 800 cases in 1997 to 26 800 cases in 2003 (i.e., from 160 to 300 cases/100 000 inhabitants) (Swedish Institute for Infectious Disease Control; http://www.smittskyddsinstitutet.se). Altered sexual behaviour may largely account for these trends, but increased use of newer diagnostic techniques, in particular nucleic acid amplification tests (NAATs), may have contributed to the higher incidence found during the last few years.

A higher sensitivity of NAATs compared to culture for the laboratory diagnosis of *C. trachomatis* has been well-documented [4,5]. Furthermore, diagnosis by NAATs of *C. trachomatis* infection in urine or vaginal specimens appears to be almost as acceptable as in urethral and endocervical samples [6–8], although reduced sensitivity with female urine samples, compared to endocervical swabs, has been reported [9,10].

The present study examined the performance of two commercial NAATs, namely the ligase chain reaction and the strand displacement assay. Both rely on the detection of a *C. trachomatis*-specific cryptic plasmid. Two venereological clinics (Lund and Helsingborg) and four clinics for adolescents participated in the study. From each patient, duplicate samples from one site only were obtained, according to the instructions of the respective manufacturer (see below). The order of sampling was changed after an interval of 2 weeks. In total, 1183 consecutive patients were investigated, with 675 endocervical swabs, 347 male urine samples and 161 male urethral samples. Patients with symptoms of genital tract infection, as well as asymptomatic cases, were included, but were not studied separately, and the laboratory did not receive any information regarding the presence or absence of symptoms. Urine samples were first void, taken in one tube and then divided into two samples.

The presence of chlamydial DNA was analysed by ligase chain reaction (LCx *Chlamydia trachomatis* assay; Abbott Laboratories, North Chicago, IL, USA) and strand displacement assay (ProbeTec Chlamydia assay; Becton Dickinson Bioscience Europe, Meylan, France), according to the manufacturers' guidelines; the LCx assay was generally performed within 2 days of sampling, whereas the ProbeTec assay was performed after 2–6 weeks, during which time the samples were kept frozen at −20°C. For both methods, a repeat positive outcome was required for the sample to be considered positive. No instances of one positive and one negative result were observed during the study.

Specimens yielding different outcomes with the two methods were stored frozen for later testing at the Department of Clinical Microbiology, University Hospital of Malmö, Sweden (K. Persson) and the Statens Serum Institute, Copenhagen, Denmark (J. Skov Jensen). In Malmö, further analysis was with the Cobas Amplicor *Chlamydia trachomatis* Test (Roche Diagnostic Systems, Branchburg, NJ, USA), targeting the same cryptic plasmid as the two assays under investigation, whereas in Copenhagen, an in-house PCR assay, targeting 16S ribosomal RNA and including an internal amplification control, was used. Aliquots of the discrepant cervical and urethral samples were referred to these external laboratories.

Among all 1183 specimens, the overall agreement between the LCx and ProbeTec assays was 98.8%, with 8.0% positive results with either method. The corresponding figures were 99.3% and 6.1% for the 675 endocervical samples, 99.4% and 9.3% for urethral samples, and 97.7% and 11.2% for urine samples (Table 1).

In total, samples from ten patients (Table 2) gave discrepant results and were tested with two other amplification assays at the external laboratories. No clinical data were used in these evaluations. Four samples were endocervical, one was urethral, and five were urine. Following further analysis, it was concluded that the the LCx assay gave five false-positive results, compared to one false-positive result with the ProbeTec assay; however, false-negative results with the ProbeTec assay were suspected in three cases, compared with one case with the LCx assay. Specimen no. 4 (Table 2) was considered to be positive, since it gave a positive result with both the LCx and Amplicor assays.

The superiority of NAATs over culture has been established in a number of previous studies. Thus, the sensitivity of culture compared with ligase chain reaction, one of the tests used in this study, was reported to be no more than 80% for endocervical samples and considerably less for urethral samples [11,12], while culture for detection of *C. trachomatis* in urine is known to be inadequate [13]. In the present study of almost 1200 consecutive patients, 98.8% agreement was obtained between the LCx and ProbeTec assays, with agreement among endocervical and urethral specimens, which required separate sampling for the two assays, being even higher than among urine specimens. The high agreement observed might be because both assays detect a common target, namely a cryptic multicopy plasmid specific to *C. trachomatis* [14]. Theoretically, this could imply a problem of specificity, in that false-positive results might be undetected, although the specificity of the ligase chain reaction has been amply documented in previous studies, suggesting that such errors are unlikely to be common [15].

Table 1. Comparison of LCx and ProbeTec assays for detection of *Chlamydia trachomatis* in different specimen types

Specimen type	Total	No. positive	Prevalence[a] (%)	Agreement (%)
Endocervix	675	41	6.1	99.3
Urethra	161	15	9.3	99.4
Urine (male)	347	39	11.2	99.7
Total	1183	95	8.0	98.8

[a]Similar figures were obtained for the LCx and ProbeTec assays.

Table 2. Analysis of discrepant samples and interpretation

Specimen/ type	ProbeTec	LCx	Copenhagen (in-house PCR)[a]	Malmö (Amplicor)[a]	Final interpretation
1/Urethra	–	+	ND/+	–/+	Positive
2/Endocervix	+	–	–/–	–/–	Negative
3/Endocervix	–	+	–/–	–/–	Negative
4/Endocervix	–	+	–/–	–/+	Positive
5/Endocervix	–	+	–/–	–/	Negative
6/Urine	– inhib	+	–	–	Negative
7/Urine	– inhib	+	–	–	Negative
8/Urine	–	+	–	–	Negative
9/Urine	– inhib	+	+	–	Positive
10/Urine	+	–	ND	+	Positive

[a]Result obtained with ProbeTec and LCx samples, respectively.
inhib, non-specific inhibition as defined by the ProbeTec guidelines; ND, not done.

Independent analysis of discrepant samples indicated one false-negative LCx result and three false-negative ProbeTec results. One of the latter samples, as well as two additional samples, showed non-specific inhibition according to the manufacturer's definition. Since an inhibition control was included in the two independent NAATs, the alternative conclusion, that the LCx assay yielded false-positive results, was less likely. However, the LCx assay was considered to yield false-positive results with five samples. It should be emphasised that all these samples were only weakly positive with the ProbeTec or LCx assays. It was concluded that repeated defrosting (on three occasions) of the samples included in the discrepant analysis did not account for the negative outcomes, since the results obtained were in agreement with the initial ProbeTec results. Overall, the rate of non-specific inhibition, limited to these three samples, was comparatively low, which was in agreement with previous reports [16,17].

In conclusion, the two NAATs, the LCx and ProbeTec assays, performed almost identically with respect to the detection of *C. trachomatis* in urethral, endocervical and urine samples. These results agree with a report from the UK [10] that showed high levels of sensitivity and specificity for both the strand displacement assay and ligase chain reaction with endocervical and urine specimens. Efficient contact tracing, combined with the use of NAATs, may help to reduce the pool of asymptomatic carriers and decrease the incidence of chlamydial genital infections. Furthermore, the

reduction in healthcare costs associated with tubal infertility, including the costs of in-vitro fertilisation, constitute an important argument for the use of DNA amplification technology for the diagnosis of *C. trachomatis*.

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RESEARCH NOTE

Human immunodeficiency virus and other sexually transmitted diseases in Cuban women

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ABSTRACT

A cross-sectional study was performed in 60 Cuban women of child-bearing age who were seropositive for human immunodeficiency virus

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